REMARKS

This case contains claims 21-22, 26, 34-35, 37, 38, 40, and 62-63 under prosecution. Claims 23-25, and 36 have been canceled without prejudice. Claims 29 and 39 have been canceled as non-elected claims. New claims (claims 61 and 62) have been added to better claim the subject matter that Applicants regard as the invention. Claims 21-22, 26, 34-35, 37, 38, and 40 have been amended for improved clarity and to better claim the subject matter which the Applicants regard as the invention. None of the amendments made herein constitutes the addition of new matter.

Invention:

The claimed invention is an immunogenic composition useful for immune protection in a CD4+ T cell independent manner. This invention was made based on the inventors' discovery that the administration of inactivated influenza virus into the CD4+ T cell deficient mice protects the animals against live influenza virus challenge. Thus, this invention provides new means of immunizing an animal deficient in CD4+ T cells. This discovery was highly unexpected because until the time of this finding, CD4+ T helper cells are believed to be essential for induction of a high affinity antibody response (Oxenius *et al.* 1998 *Adv. Immunol.* 70:313, of record herein). Without the new knowledge gained by the inventors' discovery, a person of ordinary skill in the art would not have thought of using inactivated virus to immunize an animal that is deficient in CD4+ T cells.

Claim Objections:

The patent Office alleges that the Amendment filed on April 9, 2002 introduces new matter to this case. Applicants respectfully traverse this objection for the following reasons.

The invention is the based on the inventors' new finding that administration of the inactivated influenza virus into the CD4+ T cell deficient mice protects the mice against live influenza virus challenge. Thus, the phrase, "protection in a human or animal deficient in CD4+ T cells" is not new matter. Applicants emphasize that claim 21 as amended recites the subject

matter that Applicants regard as the invention. Further, the Patent office is reminded that the results of the *in vitro* and/or *in vivo* animal studies constitute a working example if that example correlates with a disclosed or claimed invention. MPEP 2164. Applicants argue that this is the case in the present instance. The fact that the administration of inactivated influenza virus into CD4+ T cell deficient mice protects the mice from live influenza virus challenge predicts that an immunogenic composition as claimed would be useful to provide immune protection in an animal deficient in CD4+ T cells. Furthermore, a rigorous or an invariable exact correlation is not required in cases such as the present application, as stated in *Cross v. Iizuka* 753 F.2d 1040, 1050 224 USPQ 739, 747 (Fed. Cir. 1985). If the Examiner has an objective evidence as to why the invention as claimed would not work, such evidence has not been presented to the Applicants.

Claim rejections under 35 U.S.C. § 112:

Claims 21-26, 34-38, and 40 stand rejected under 35 U.S.C. § 112, second paragraph for allegedly vague and indefinite. Applicants respectfully traverse this rejection.

Claim 21 has been amended to specify the term, "a sialic acid binding component" as hemagglutinin. Claim 21 has been further amended to recite an inactivated virus only. The term "inactivated" is a well known term in the art and is intended to indicate that the virus is non-infectious as indicated in Example 2 on page 20, lines 9-11.

Claims 23-25 and 36 have been canceled without prejudice.

Claim 34 has been amended to recite that "a sialic acid binding component" be hemagglutinin.

Claim Rejections under 35 U.S.C. § 112:

Claims 21-26, 34-38, and 40 are rejected under 35 U.S.C. § 112, first paragraph, on the ground that the Specification allegedly does not provide enablement for a person of ordinary skill in the art to make and use the invention commensurate in scope with the claims.

The claimed invention is an immunogenic composition useful for providing CD4+ T cell independent immune protection. The Specification discloses an example using inactivated influenza virus. It is well known in the art that binding of influenza virus to cell surfaces requires sialic acid receptors and further that influenza virus can bind to heterotypic antigens (i.e., other virus particles) in a sialic acid dependent manner (see Compans, R. (1974) J. Virol.14:1307-1309, submitted herewith as Exhibit A). Accordingly, it is submitted that the claims as amended are enabled, i.e., a person of ordinary skill in the art can make and use the invention. Withdrawal of the rejection under 35 U.S.C. § 112 is respectfully requested.

Claim Rejections under 35 U.S.C. § 102:

Claims 21-26, 34-38, and 40 are rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by Compans (US Patent No. 4,790,987). Applicants respectfully traverse this rejection.

The cited patent teaches a composition having a certain glycoprotein complexed with a lipid and a pharmaceutically acceptable carrier. The Examiner is correct that the virus from which the glycoprotein is derived can be the same virus, i.e., influenza virus. However, this patent does not teach a composition containing an inactivated intact virus and a sialic acid binding component as claimed in the present application. Thus, the claimed invention is not anticipated by the cited patent.

Claims 21-25, 34-38, and 40 are rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by Pertmer et al. (J. Virol 1996, 70: 6119-6125). Applicants respectfully traverse this rejection.

Pertmer et al. determined the differential immune responses of DNA vaccination by administering via various delivery routes. The DNA construct used therein is an influenza virus nucleoprotein expression vector. Inactivated influenza virus was used to immunize the mice as a control. The claimed invention is not anticipated by this reference. There is no teaching that

immune response elicited by the inactivated influenza virus was CD4+ T cell independent. It was known that inactivated viruses are immunogenic although the response was weak. There was no reason for a skilled artisan to predict that the immune response by the administration of the inactivated influenza virus would be CD4+ T cell independent.

Claims 21-26, 34-38, and 40 are rejected under 35 U.S.C. 102(b) as being allegedly anticipated by Muster et al. (J. Virol 1994, 68:4031-4034). Applicants respectfully traverse this rejection.

Muster et al. teaches that a stretch of six amino acids derived from HIV gp41 is sufficient to generate neutralizing antibodies specific for the peptide when expressed as chimeric hemagglutinin molecule by inserting in the loop of antigenic site B of the influenza virus hemagglutinin in OF-1 mice. In these studies, the virus used was a live recombinant influenza virus (not inactivated) and the hemagglutinin molecule was modified. This reference does not teach the claimed composition containing an inactivated virus and a sialic acid binding component, useful for providing immune protection in an animal deficient in CD4+ T cells.

Claims 21-25, 34-38, and 40 are rejected under 35 U.S.C. 102(b) as being allegedly anticipated by Pales et al. (J. Inf. Dis. 1997, 176:S45-S49). Applicants respectfully traverse this rejection.

As correctly pointed out by the Examiner, Pales et al. discusses a general strategy of how to use live influenza virus that has been genetically engineered to express a foreign antigen to enhance immune response. There is no teaching of the claimed invention.

Claims 21-25, 34-38, and 40 are rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by Li et al. (J. Virol. 1993, 67:6659-6666). Applicants respectfully traverse this rejection.

Li et al. discloses live chimeric influenza virus that has been genetically engineered to express a foreign antigen as an expression vector for inducing B- and T-cell-mediated immunity against other infectious agents. The allegation that the claimed composition is the same as that of Li et al. is not correct. The claimed composition contains inactivated virus and a sialic acid binding component useful to provide immune protection in a CD4+ T cell independent manner.

Claims 21-25, 34-38, and 40 are rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by Chiba et al. (Arch. Virol. 1999, 144:1469-1485). Applicants respectfully traverse this rejection.

As pointed out by the Examiner, Chiba et al. discloses a recombinant vaccinia virus expressing a chimeric protein of influenza HA containing a stretch of 15 amino acids of HIV gp160. When this virus was administered to BALB/c mice, it primed CD8+ T cells but failed to elicit antibody specific for the HIV sequence. This reference does not teach a immunogenic composition containing an inactivated virus and a sialic acid binding component that is useful for immune protection in a CD4+ T cell independent manner.

Applicants emphasize that the anticipating references must teach each and every element of the claimed invention. MPEP2131. Further, it is stated in *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989), "The identical invention must be shown in as complete detail as is contained in the.....claim." Applicants argue that none of the cited art teaches the invention i.e., the invention can not be anticipated by any of the cited art. The cited references disclose the use of a live, modified virus. None of the cited art teaches the utility of a composition for an animal deficient in CD4+ T cells. Accordingly, withdrawal of the rejection under 35 U.S.C. 102 is respectfully requested.

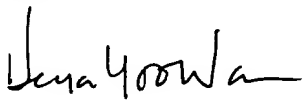
Conclusion

Based on the foregoing amendments and the arguments, it is submitted that this case is in condition for allowance and passage to issuance is respectfully requested.

If there are any outstanding issues related to patentability, the courtesy of a telephone interview is requested, and the Examiner is invited to call to arrange a mutually convenient time.

This amendment is accompanied by a Petition for Extension of Time (one month) and a check in the amount of $55.00 as required under 37 C.F.R. 1.17(a)(2) for a small entity. If the amount submitted is incorrect, however, please charge any deficiency or credit any overpayment to Deposit Account No. 07-1969.

Respectfully submitted,



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21. (Twice amended) An immunogenic composition useful for providing immune protection in [a human or] an animal deficient in CD4+ T cells, comprising a sialic acid binding component and an inactivated [or attenuated] target [cell or] virus[.] wherein said sialic acid binding component is a hemagglutinin.

22. (Once amended) The immunogenic composition of claim 21 wherein [said sialic acid binding component] said hemagglutinin is from [a hemagglutinin of] an inactivated orthomyxovirus or a paramyxovirus.

26. (Twice amended) The immunogenic composition of claim [25] 21 wherein said target virus [preparation] is selected from the group consisting [a preparation] of simian immunodeficiency virus, human immunodeficiency virus, feline immunodeficiency virus, or bovine immunodeficiency virus, rabies virus, measles virus, vesicular stomatitis virus, flavivirus, alphavirus or herpes virus.

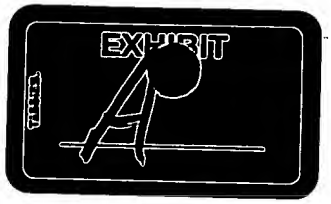
34. (Twice amended) An immunogenic composition comprising a sialic acid binding component and an antigen of [a target cell or] an inactivated target virus[.] wherein said sialic acid binding component is a hemagglutinin.

35. (Once amended) The immunogenic composition of claim 34 wherein [the sialic acid binding component is a] said hemagglutinin [of] is from an inactivated orthomyxovirus or paramyxovirus.

37. (Twice amended) The immunogenic composition of claim 34 wherein the antigen of a target [cell or target] virus comprises sialic acid or polymerized sialic acid.

38. (Once amended) The immunogenic composition of claim 37 wherein the [at least one] antigen of a target [cell or target] virus is comprised within inactivated [or attenuated target cell or inactivated or attenuated] target virus or virus-like particles of a target virus.

40. (Once amended) The immunogenic composition of claim [38] 34 wherein the target virus is simian immunodeficiency virus, human immunodeficiency virus, feline immunodeficiency virus, or bovine immunodeficiency virus, rabies virus, measles virus, vesicular stomatitis virus, flavivirus, alphavirus or herpes virus.



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Hemagglutination-Inhibition: Rapid Assay for Neuraminic Acid-Containing Viruses

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Influenza virus particles bind rapidly to vesicular stomatitis, Sindbis, or Rauscher murine leukemia virus particles, forming mixed aggregates demonstrable by electron microscopy. The normal hemagglutinating property of influenza virus is inhibited by these viruses, providing a rapid quantitative assay. Prior treatment with neuraminidase blocks the ability of other viruses to inhibit influenza virus hemagglutination.

Since the discovery of hemagglutination by influenza virus in 1941 (5, 15), this phenomenon has been useful in providing a rapid and convenient means of quantitation of virus particles. In addition, hemagglutination inhibition (HI) by antiviral antibody has provided an excellent method for quantitation of antibody titers, and for analysis of antigenic differences among virus strains. Whereas the hemagglutinating property of influenza, parainfluenza and certain other viruses can be observed without difficulty, in many other groups of viruses hemagglutination requires rather precise control of temperature, pH, and other variables in order to obtain reproducible results, and very low titers are observed with many virus strains. Thus a rapid, simple assay is unavailable for many viruses.

Most enveloped viruses which form by budding at the cell surface contain neuraminic acid as a component of glycoproteins and glycolipids (1, 10, 12, 16, 17), with the exception of myxo- and paramyxoviruses which contain neuraminidases and therefore lack neuraminic acid (9, 8, 11). The latter viruses bind specifically to neuraminic acid-containing receptors on cell surfaces (3, 7). Recently it has been found that temperature-sensitive mutants of influenza virus which lack neuraminidase do contain neuraminic acid in progeny virus particles, which form extensive aggregates (P. Palese, K. Tobita, M. Ueda, and R. W. Compans, Virology, in press). Disaggregation occurs upon treatment with neuraminidase, indicating that neuraminic acid in virions is serving as the attachment site for adjacent virions. It therefore seemed likely that neuraminic acid-containing components of other viruses might also serve as receptors for influenza virus hemagglutinin.

The WSN strain of influenza virus and the parainfluenza virus SV5 were grown in MDBK cells (2), purified as described previously (13), and dialyzed against 0.01 M phosphate buffer, pH 7.2. Vesicular stomatitis virus (VSV) (Indiana, New Jersey, and Cocal strains) and Sindbis virus were grown in BHK21-F cell monolayers and purified by the same procedure. Rauscher murine leukemia virus was purified by the same procedure from culture media of infected BALB-3T3 or JLS-V9 cells. Determination of protein concentrations of purified viruses was done by the Lowry procedure (14).

Aggregation of influenza virions with neuraminic acid-containing virions of other major groups could readily be observed by electron microscopy. Figure 1 shows an aggregate of influenza and VSV particles, and similar aggregates were observed upon mixing influenza virions with Sindbis or Rauscher leukemia virions (Fig. 2). The size of the aggregates increased with time, and aggregates containing hundreds of particles were frequently observed.

It seemed likely that the binding of heterologous virions to surfaces of influenza virions would inhibit hemagglutination by the latter, providing the basis for a rapid assay for neuraminic acid-containing viruses, many of which hemagglutinate only under very precise conditions (4, 6, 18, 19). The use of HI to measure the time course of appearance of VSV in BHK21-F cell cultures after single-cycle infection is shown in Fig. 3. The rise in HI titer parallels the rise in infectious virus, which was determined separately by plaque assay on BHK21-F cell monolayers. Reading the HI titer as the reciprocal of the highest dilution causing detectable inhibition of hemagglutination, a maximal HI titer of 1,024 U/ml was reached at 10 h, whereas control cells incubated for this length of time showed little or no HI activity in the culture medium.

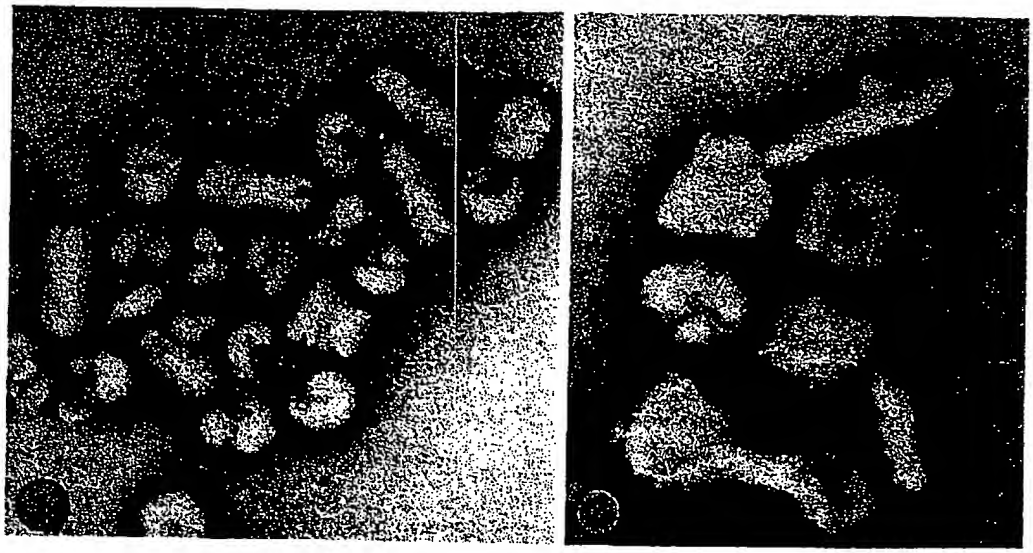

FIG. 1. *Aggregate of bullet-shaped VSV (Indiana) and roughly spherical influenza virions observed 10 min after mixing samples of purified virus particles. Sodium phosphotungstate stain. Magnification: ×85,000.*

FIG. 2. *Aggregate of two spike-covered influenza virions and three Rauscher murine leukemia virus particles. Sodium phosphotungstate stain. Magnification: ×120,000.*

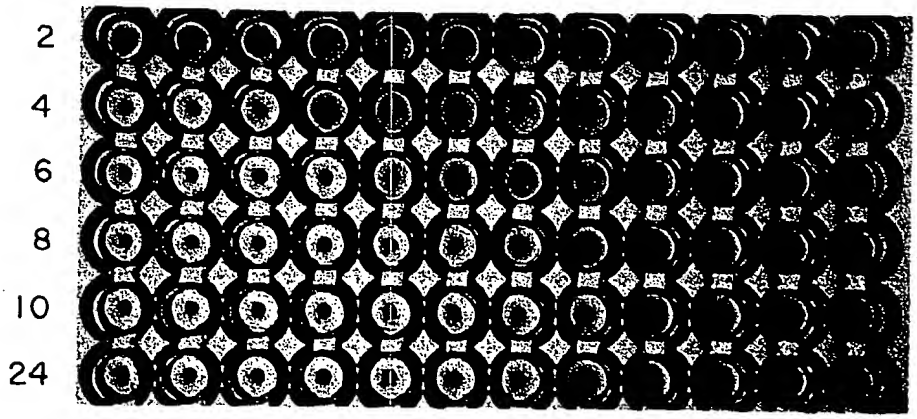

FIG. 3. *Assay of growth of VSV (Indiana strain) in BHK21-F cell cultures by HI. Cells were infected at a multiplicity of 50 PFU/cell, and after 1 h the inoculum was removed and replaced with reinforced Eagle medium. At the indicated times (hours postinfection), culture medium was harvested and portions were diluted serially (twofold) in phosphate-buffered saline in a Lindbro microtiter plate. To 50 μliters of each dilution was added 50 μliters of purified influenza virus, previously diluted in phosphate-buffered saline to a concentration of 12 hemagglutinating U/ml. The hemagglutination titer of the influenza virus was determined in a microtiter plate by serially diluting virus in phosphate-buffered saline and adding 100 μliters of a 0.36% chicken red blood cell suspension to 100 μliters of each dilution. Titers were read after 60 min, and the highest dilution showing marked or complete agglutination (2 or 3+) was considered as the end point. Mixtures of VSV and influenza virus were held for 30 min at 20 C, and 100 μliters of a 0.36% suspension of chicken red blood cells in phosphate-buffered saline was added. The titer was read after 60 min at 20 C, and the highest dilution causing detectable inhibition of hemagglutination was defined as containing 1 HI U/ml.*

There are approximately 10^7 PFU of VSV per HI unit under the conditions described for Fig. 3. Thus it is feasible to determine relative amounts of VSV in culture media by HI titrations without plaque assay, concentration, or purification of virus.

Enveloped viruses of several major groups were assayed for HI titers as described in Fig. 3,

and possessed activities comparable to that of VSV. Using purified virions, HI titers of Sindbis, Rauscher leukemia virus, and the Cocal, New Jersey, and Indiana strains of VSV were determined and all fell in the range of 2,000 to 10,000 HI U per mg of virus protein. In contrast, the parainfluenza virus SV5, which contains no neuraminic acid on its surface, is devoid of HI activity.

To determine conditions for obtaining maximal sensitivity of the assay, virus mixtures were held at 4 or 20 C for intervals from 15 min to 2 h before addition of red blood cells. With VSV the maximal titer was reached after 30 min at 20 C, whereas Sindbis virus required a 2-h preincubation at 20 C and Rauscher leukemia virus, 2 h at 4 C for maximal titers, which were two- or four-fold higher than those obtained by incubation for 30 min at 20 C. The WSN strain of influenza virus possesses low neuraminidase activity, which accounts for its lack of interference with the assay when virus mixtures or virus-red cell suspensions were incubated at 20 C.

To further demonstrate that neuraminic acid on the surfaces of virus particles is required for HI activity, virions were treated with neuraminidase (protease free, Behringwerke). Purified VSV (Indiana) was incubated for 2 h with 5 U of neuraminidase per ml. Measurement of HI activity was done at 4 C to prevent interference by neuraminidase. The HI titer of a control sample was 2,048 U/ml, whereas the titer of the neuraminidase-treated sample was 8 U/ml. Neuraminidase treatment of Sindbis and Rauscher leukemia virus produced similar inhibition of HI activity.

The present results demonstrate that inhibition of influenza virus hemagglutination is a potentially useful quantitative assay for neuraminic acid-containing viruses of several major groups. Measurement of virus titers in cell culture fluids, or fractions from purification procedures by this assay, obviously requires suitable control experiments since many neuraminic acid-containing substances act as inhibitors of influenza virus hemagglutination. In this regard, 2 percent calf serum or fetal calf serum, or 15% sucrose or potassium tartrate, had no inhibitory effect under the conditions described for Fig. 3.

Preliminary results indicate that blocking of HI by specific antibody may provide a useful method for determination of antibody titers, and for identification of serological specificity of viral surface antigens.

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